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TITLE: Immune Response Genotypes and Risk of Young Adult Hodgkin Lymphoma

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14. ABSTRACT
Hodgkin lymphoma (HL) is the first and second most common cancer among young women and men 20-39 years old, respectively. Our previous results from a small twin study suggested that an inherited imbalance in the immune response to infection could increase susceptibility to young adult HL. Here we will further test the hypothesis that the susceptible immuno-phenotype for HL is determined by a genetic tendency toward an exaggerated Th2 and/or inflammatory response and/or a depressed Th1 response, resulting from genotypes that regulate these responses. After 10 months of harmonizing the IRB materials between USC and the DOD, we have begun data collection. In the last month, we identified the first 25 young adult HL patients from the L.A. cancer registry and of those, have obtained and processed blood samples for DNA from 12 patients plus their parents to serve as controls. We will begin genotyping when we collect the projected 368 cases plus their parents or siblings (controls). If we can identify the immune pathways responsible for this cancer, we may be able to design immunotherapy to prevent it, avoiding many lost years of productivity and loss of life that results from the treatment and its complications.

15. SUBJECT TERMS
Hodgkin lymphoma, immune response, Th1, Th2, cytokines, genetic, epidemiology, risk factors

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INTRODUCTION

BODY

The grant was awarded and funded late because this proposal was an alternate from 2005, and the funds only because available in September of 2006. We received the funding in October of 2006 so we were a month or so late in getting started. The study materials (questionnaires, informed consents, HIPAA) and database were developed. It took 10 months for the two IRBs (DOD and USC) to come to an agreement over the injury statement in the informed consent. Once that was finished, we received the case listing of newly diagnosed Hodgkin lymphoma patients in Los Angeles County.

KEY RESEARCH FINDINGS

There are no key research findings to date.

CONCLUSIONS

There are no research conclusions based on this DOD supported proposal to date. Because of the delay due to late notification of the award and late funding and the length of time it took to harmonize the injury statement for the informed consent, we are about 6 months behind schedule. If we can keep pace with our first month, we can obtain DNA samples from 12 patients plus family controls each month, we can finish data collection in 30 months (about six months later than we originally planned). Since there are 36 months left in the grant, we will then finish the genotyping and analysis of the samples during the last 6 months of the proposal in Year 4.

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INTRODUCTION

There is strong evidence supporting the hypothesis that the young adult form of HL is caused by an aberrant immune response to a common childhood infection (as yet unknown), acquired relatively late (in adolescence or young adulthood)[1]. There is additional evidence for a genetic contribution which may predispose to that aberrant immune response[2]. We postulate that the genetic mechanism might involve a heritable propensity to produce higher levels of Th2/inflammatory cytokines, and lower levels of Th1 cytokines[3,4]. This hypothesis is difficult to test directly since the disease alters the immune response. However, the production of these cytokines is governed in part by polymorphic genes, with functional variants responsible for some inter-individual variation[5,6]. These genetic variants are immutable and can be studied at point in the disease process or at any phase of life.

Preliminary studies of twins with young adult HL support this hypothesis and suggest that genotypes associated with increased Th2 and inflammatory cytokines and decreased Th1 cytokines increase risk of young adult HL[3,4]. Confirmation of these results in a population-based study is necessary. Cohort studies are impractical since cohorts would have to be designed to enroll individuals by adolescence in order to ensure that they were identified prior to peak occurrence of this cancer. In addition, the cancer is relatively rare and there are not enough cases within the appropriate age range in existing cohorts.

Case-control studies are also problematic. It is increasingly difficult to recruit a representative group of controls due to a decline in participation rates and an increase in barriers to recruitment (such as caller ID, use of cell phones, etc)[7]. Fortunately, a class of study designs based on the transmission disequilibrium test (TDT) eliminates some of these problems altogether and minimizes the others[8]. This approach uses a data structure of case-parent trios in which genotype-disease associations are measured by comparing genotypes in the case with those that could have been transmitted by his/her parents. Refinements in the method allow for enrollment of incomplete trios (e.g. case and one parent or case and one parent plus a sibling, etc.)[9], although these other relative sets provide reduced power. The statistical and genetic properties of the resulting estimators have been extensively validated and shown to work well for diseases with sufficiently young age-at-onset to allow for enrollment of parents. We predict that this design will be appropriate to study genetic risk factors for this form of cancer since the targeted age group is relatively young (15-45 years old). Thus, we were awarded this grant proposal to test the following hypothesis and to achieve the following Technical Objectives in a 4-year study/

HYPOTHESES

1) To test the hypothesis that genotypes associated with increased secretion of T helper cell 2 cytokines and/or decreased secretion of T helper 1 and T regulatory cell cytokines are associated with an increased risk of young adult Hodgkin lymphoma [HL].
2) To test the hypothesis that variations in genotypes of other immune response elements (antigen processing proteins and other regulatory molecules) are associated with an increased risk of young adult Hodgkin lymphoma.
3) To determine if these associations vary by histologic subtype of Hodgkin lymphoma.
4) To determine if these associations vary by the presence or absence of Epstein-Barr virus (EBV) in the tumor.

TECHNICAL OBJECTIVES

1) To identify, enroll and collect blood specimens from 368 adolescents and young adults 18-to 45 years old diagnosed with Hodgkin lymphoma, and from their parents, in a case-parent-trio study.
2) To genotype 1,536 SNPs of genes encoding cytokines, their receptors, antigen processing genes, and other immune response factors using the Illumina genotyping platform.
3) To conduct a transmission disequilibrium test and conditional logistic statistical modeling to test the association between genotypes and young adult Hodgkin lymphoma risk.
4) To collect tumor tissue from cases to validate histological subtype of Hodgkin lymphoma.
5) To collect tumor tissue from cases to determine the presence or absence of EBV.
6) To analyze the statistical data resulting from the genotyping.

BODY

The grant was awarded and funded late because this proposal was an alternate from 2005, and the funds only because available in September of 2006. We received the funding in October of 2006 so we were a month or so late in getting started. The study materials (questionnaires, informed consents, HIPAA) and database were developed. It took 10 months for the two IRBs (DOD and USC) to come to an agreement over the injury statement in the informed consent. Once that was finished, we received the case listing of newly diagnosed Hodgkin lymphoma patients in Los Angeles County and commenced with the study. We received a listing of 83 patients diagnosed within the last year while residents of Los Angeles County. According to our study protocol, letters were sent to the physicians of the first 25 patients to notify them that we intended to contact their patients in case they knew of a reason we should not (i.e. patient deceased, too ill etc). If we do not hear any response from the physician within 2 weeks, we then send a recruitment letter to patients. There was no information on location or address for 3 patients so we are in the process of tracing them to try and obtain new contact information. We sent recruitment letters to the remaining 22 patients. Of these, we obtained blood and saliva samples and completed questionnaires from 13. Of the 13 participating patients, we obtained blood and saliva samples from parents and/or siblings (for use as controls) from all but one patient. (That patient did not want her family members contacted). Questionnaires have been entered for all of these subjects (patients and family members) and pathology reports have been reviewed and submitted to the Population Tissue Retrieval Program for collection of tumor slides from patients (for Epstein-Barr virus testing). Upon receipt of 25 tumors, we will send these to our consultant Dr. Lawrence Weiss at City of Hope to test for the virus. A summary of the completed tasks from the proposal Statement of Work is given below.

Task 1: To prepare and submit IRB documents (protocol detail, physician, patient and parent letters, HIPAA forms, tumor block release forms and questionnaires). (months -3 to 1, i.e. begun three months before grant period begins and completed by the grant start date). **COMPLETE**

Task 2: To develop a tracking database to log participants and non-participants, and keep track of interview and specimen collection dates (months -3 to 1, i.e. begun three months before grant period begins and completed by the grant start date). **COMPLETE**

Task 3: To identify patients 18-45 years old diagnosed with Hodgkin lymphoma from October 1, 2005 through March 31, 2009 while living in Los Angeles County, California within three months of diagnosis through rapid reporting by the USC Cancer Surveillance Program (months 1-42). **IN PROGRESS**

Task 4: To contact and recruit 368 (75%) of these patients for the study (months 1-42) **IN PROGRESS**

Task 5: To contact and recruit parents of these patients for the study (months 1-42) **IN PROGRESS**

Task 6: To obtain blood specimens and questionnaires on 368 patients and their parents (months 1-42) **IN PROGRESS**

Task 7: To request tumor tissue blocks of the 368 enrolled patients from hospitals through the USC Slide/Block Retrieval Program (PI directs the program). We estimate that we can obtain 80% of the tissue blocks requested. (months 3-45) **IN PROGRESS**

Task 8: To section tumor tissue blocks and stain 1 of the 10 resulting slides with H & E and review the histology for verification of diagnosis (months 3-45). **IN PROGRESS**

Task 9: To determine the presence or absence of Epstein-Barr virus in the tumor cells using in situ hybridization. (months 3-45). **NOT YET BEGUN**

Task 10: To process blood specimens from subjects including collection and storage of serum, isolation of buffy coat and extraction of DNA (months 1-42). **IN PROGRESS**

Task 11: To aliquot extracted DNA into 96-well plates to prepare for genetic analysis and perform genotyping of 1,536 single nucleotide polymorphisms from extracted DNA from all subjects using the Illumina assay platform (month 43). **IN PROGRESS**

Task 12: To perform statistical analysis of the data to determine which genotypes are associated with young adult Hodgkin lymphoma risk. (months 44-47). **NOT YET BEGUN (ON TRACK)**

Task 13: To write a paper summarizing the findings (month 48). **NOT YET BEGUN (ON TRACK)**

Task 14: To produce annual reports summarizing the progress of the grant (depending on the requirements, months 12, 24, 36, 48). **12-MONTH REPORT SUBMITTED**

KEY RESEARCH ACCOMPLISHMENTS

There are no key research findings to date.

REPORTABLE OUTCOMES

There are none to date.

CONCLUSIONS

There are no research conclusions based on this DOD supported proposal to date. Because of the delay due to late notification of the award and late funding and the length of time it took to harmonize the injury statement for the informed consent, we are about 6 months behind schedule. If we can keep pace with our first month, we can obtain DNA samples from 12 patients plus family controls each month, we can finish data collection in 30 months (about six months later than we originally planned). Since there are 36 months left in the grant, we will then finish the genotyping and analysis of the samples during the last 6 months of the proposal in Year 4.

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APPENDICES

None